

Environmental *Shewanella xiamenensis* Strains That Carry *bla*_{OXA-48} or *bla*_{OXA-204} Genes: Additional Proof for *bla*_{OXA-48-Like} Gene Origin

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The chromosome-encoded beta-lactamases of *Shewanella* spp. have been recognized as progenitors of *bla*_{OXA-48-like} genes (1). The analysis of available genome sequences of *Shewanella* spp. showed the presence of *bla*_{OXA-48-like} genes in their chromosome with at least 80% identity to *bla*_{OXA-48} (2). Although initially considered geographically restricted, it has now been demonstrated that the spread of the *bla*_{OXA-48} gene is one of the greatest concerns in terms of antibiotic resistance (3). In fact, since its first description less than a decade ago (4), *bla*_{OXA-48-like} genes have been reported worldwide (1, 3). Several variants of *bla*_{OXA-48} genes have been identified in *Enterobacteriaceae* strains, mostly isolated from clinical settings. So far, *bla*_{OXA-181} (5) and *bla*_{OXA-48b} and *bla*_{OXA-199} (2) have been reported in *Shewanella xiamenensis* strains.

The OXA-204 enzyme was recently described in *Klebsiella pneumoniae* clinical isolates in Tunisia. Its substrate profile is similar to that of OXA-48, from which differs by only two amino acids (6). The origin of *bla*_{OXA-204} was not identified before. Here we report the isolation from river water in Portugal of three *S. xiamenensis* strains, one of which carried the *bla*_{OXA-204} gene. Strains IR24, IR33, and IR34 were isolated from rivers (7) in MacConkey agar plates supplemented with 8 µg/ml of imipenem and identified by 16S rRNA gene sequencing as *S. xiamenensis*. Sequencing of the *bla*_{OXA-48-like} genes amplified by PCR using previously described primers (2) revealed that these strains carried either a *bla*_{OXA-48b} gene (IR24 and IR33) or a *bla*_{OXA-204} gene (IR34). Antimicrobial susceptibility and MICs were determined in Mueller-Hinton agar plates at 37°C and interpreted according to the CLSI guidelines (8). Results are shown in Table 1. All three isolates were resistant to penicillins and carbapenems but susceptible to expanded-spectrum cephalosporins and fluoroquinolones. MICs of ertapenem, imipenem, and meropenem for the OXA-204-producing strain were at least 4 times higher than those determined for the OXA-48-producing strains. Moreover, MICs for carbapenems were also higher than those previously described for *K. pneumoniae* carrying *bla*_{OXA-204} (6).

To investigate the genetic context, primers were designed targeting regions commonly described as flanking *bla*_{OXA-48-like} genes in *Shewanella* spp. (2): upstream, a gene encoding peptidase C15 (C15_fwd [5'-TTACGGCCTGGGAAGTGTTC-3']), and downstream, the *lysR* gene (*lysR_rev* [5'-AAGGGATTCTCCCAAGCT

GC-3']), which codes for a putative LysR transcriptional regulator. Sequencing of the amplified region revealed identical contexts for both *bla*_{OXA-204} and the *bla*_{OXA-48} genes, presenting upstream the C15 gene and downstream the *lysR* gene. This constitutes the first report on *S. xiamenensis* strains carrying a *bla*_{OXA-204} gene, suggesting that the emergence of different *bla*_{OXA-48-like} genes probably had its origin in different *S. xiamenensis* strains. It also suggests the participation of diverse mobilization events and mechanisms in the transfer of *bla*_{OXA-48-like} genes from *Shewanella* spp. to *Enterobacteriaceae*. Whereas *ISEcp1* has been identified preceding the *bla*_{OXA-204} and *bla*_{OXA-181} gene, *IS1999* has been found upstream of *bla*_{OXA-48} genes (1). Moreover, it is of great relevance to acknowledge that these genetic events may have occurred in natural environments, reinforcing the idea of the importance of aquatic systems in the evolution and spread of antibiotic resistance.

Nucleotide sequence accession numbers. The nucleotide sequence data determined in this work have been deposited in GenBank under accession numbers [KC902850](#), [KC902851](#), and [KC902852](#).

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REFERENCES

- Poirel L, Potron A, Nordmann P. 2012. OXA-48-like carbapenemases: the phantom menace. *J. Antimicrob. Chemother.* 67:1597–1606.
- Zong Z. 2012. Discovery of *bla*_{OXA-199}, a chromosome-based *bla*_{OXA-48-like} variant, in *Shewanella xiamenensis*. *PLoS One* 7:e48280. doi:10.1371/journal.pone.0048280.

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TABLE 1 Resistance phenotype and MICs of carbapenems for *S. xiamenensis* strains^a

Strain:: <i>bla</i> _{OXA-48-like} gene	Resistance phenotype	MIC in µg/ml (resistance status)		
		ERT	IPM	MER
<i>S. xiamenensis</i> IR24:: <i>bla</i> _{OXA-48-like}	AML, AMC, IPM, ERT, ATM	8 (R)	4 (R)	2 (I)
<i>S. xiamenensis</i> IR33:: <i>bla</i> _{OXA-48}	AML, AMC, CTX, IPM, ERT, ATM	8 (R)	4 (R)	1 (S)
<i>S. xiamenensis</i> IR34:: <i>bla</i> _{OXA-204}	AML, AMC, IPM, ERT, NAL	>32 (R)	>32 (R)	8 (R)

^a AML, amoxicillin; AMC, amoxicillin plus clavulanic acid; ATM, aztreonam; CTX, cefotaxime; ERT, ertapenem; IPM, imipenem; MER, meropenem; NAL, nalidixic acid; I, intermediate; S, susceptible; R, resistant.

3. Patel G, Bonomo RA. 2013. “Stormy waters ahead”: global emergence of carbapenemases. *Front. Microbiol.* 4:48.
4. Poirel L, Heritier C, Tolun V, Nordmann P. 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 48:15–22.
5. Potron A, Nordmann P, Lefeuvre E, Maskari ZA, Rashdi FA, Poirel L. 2011. Characterization of OXA-181, a carbapenem-hydrolyzing class D β -lactamase from *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 55:4896–4899.
6. Potron A, Nordmann P, Poirel L. 2013. Characterization of OXA-204, a carbapenem-hydrolyzing class D β -lactamase from *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 57:633–636.
7. Tacão M, Correia A, Henriques I. 2012. Resistance to broad-spectrum antibiotics in aquatic systems: anthropogenic activities modulate the dissemination of *bla*CTX-M-like genes. *Appl. Environ. Microbiol.* 78:4134–4140.
8. CLSI. 2012. Performance standard for antimicrobial susceptibility testing—document approved standard M100-S22. CLSI, Wayne, PA.